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Direct measurement of diffusing proteins in nanochannels using fluorescence correlation spectroscopy

N.F.Y. Durand[a]*, C. Dellagiacoma[b], R. Goetschmann[a], A. Bertsch[a], I. Märki[b], T. Lasser[b], P. Renaud[a]

[a]*Microsystems Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland*
[b]*Biomedical Optics Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland*

Abstract

Transport of proteins in liquid-filled nanometer-sized apertures with charged surfaces based on both diffusion and convective flow is investigated with the use of fluorescence correlation spectroscopy. The measured fluctuations of fluorescence allow the evaluation of the concentration of molecules directly inside the nanochannel. Here, we supply theoretical models describing the concentration profile of proteins inside nano-confinements, taking into account the steric exclusion, the presence of reversibly adsorbed molecules on surfaces and the exclusion-enrichment effect due to interaction with the electrical double layer. Therefore, this study presents a new step for a deeper understanding of the transport of molecules in nanofluidics.

Keywords: Diffusion; Fluorescence correlation spectroscopy; Nanochannel; Nanofluidics; Protein; Wheat germ agglutinin

1. Introduction

Micro and nanosystems have already generated considerable activity, economically and scientifically, and their importance is expected to increase significantly in the next decades. This development towards miniaturization results mainly from an expanding need for cleaner, cheaper and faster diagnostic devices. As the surface-to-volume ratio increases while scaling down, the hydrodynamic properties and the liquid behavior considerably change as compared to bulk liquids. The surface charge becomes vitally important, as a significant fraction of the total charge is bound to the walls. This phenomenon, among others alters the diffusion of charged species and in particular of proteins and until today no direct measurements were performed in nano-confined spaces in order to study their transport behavior.

In this article, we present new theoretical models describing the concentration profile of proteins inside a 1D nanochannel. Based on single molecule detection experiments by fluorescence correlation spectroscopy (FCS), we report the different steady-state concentration profiles governed by diffusion, by convective flow and by the solution characteristics (ionic strength, pH, charge and concentration of proteins, etc.).

* Corresponding author. Tel.: +41 21 693 67 52; fax: +41 21 693 59 50.
E-mail address: nicolas.durand@epfl.ch

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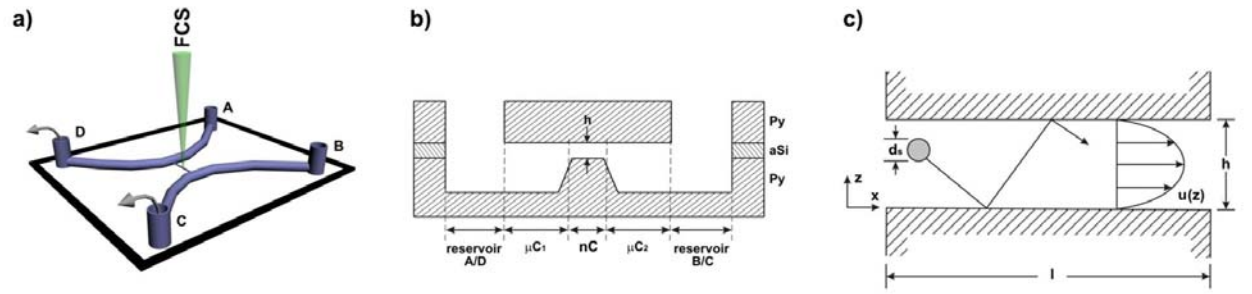

Fig. 1. (a) 3D schematic of the nanofluidic system (not to scale) made of 2 microchannels (μC_1 and μC_2) linked together by a nanochannel of height h=50 nm. The FCS measurements are performed directly in the nanochannel. (b) cross section of the nanochannel and the microchannels (not to scale). A 50 nm amorphous silicon layer (aSi), sandwiched between 2 Pyrex wafers (Py), defines the height of the nanochannel. (c) Cross section of the nanochannel containing a spherical solute of diameter d_s. A positif convective flow *u(z)* is schematically depicted.

2. Hindered diffusion of proteins in nano-confined spaces

For solutes that are large enough to behave as hydrodynamic particles, the constrained space of a nanochannel causes the molecular friction coefficient to exceed its maximum value and leads to steric exclusion. The equilibrium partitioning Φ_{nC} of solute concentration between inside and outside a 1D nanochannel, taking into account adsorption of solute on surfaces[1], steric exclusion[2] and electrostatic interactions[3], is given by[4]:

$$\Phi_{nC} = \frac{\langle c_{nC} \rangle}{c_0} = \frac{\chi}{h} \int_0^{h(1-\lambda)} e^{-\frac{q\Psi(z)}{k_B T}} dz \tag{1}$$

where $\langle c_{nC} \rangle$ is the mean concentration of solute in the nanochannel and c_0 the concentration of solute in the bulk. λ is defined as the ratio of the solute diameter d_s to nanochannel height h ($\lambda = d_s/h$). k_B is the Boltzmann constant, T the temperature, q the net charge of the solute and $\Psi(z)$ is the Debye-Hückel approximation for the electrical potential between two infinite planar surfaces[5]:

$$\psi(z) = \zeta \frac{\cosh((h/2 - z)/\lambda_D)}{\cosh(h/2\lambda_D)} \tag{2}$$

where ζ is the zeta potential and λ_D the electrical double layer (EDL) thickness corresponding to the Debye screening length, in a 1:1 electrolyte solution[3]. We defined the bulk-surface partitioning cofactor χ as:

$$\chi = 1 + \frac{2\Gamma_{max}}{N_A \cdot h} \cdot \frac{K}{K \cdot c_0 + 1} \tag{3}$$

where N_A is the Avogadro constant, Γ_{max} the full coverage surface concentration, K the equilibrium constant of adsorption and desorption. In the experiments performed in this study, the solute is not charged since the pH is equal to the isoelectrical point (pI) of proteins. The proteins charge q is zero and equation 1 is reduced to:

$$\Phi_{nS} = (1-\lambda)\left(1 + \frac{2\Gamma_{max}}{N_A \cdot h} \cdot \frac{K}{K \cdot c_0 + 1}\right) \tag{4}$$

This explains why the effective concentration inside the nanochannel is higher than the one in the microchannel.

3. Experimental

As presented in Fig. 1, the device used in the experiment consists of 2 microchannels bridged by a thin nanochannel (height h=50 nm, width w=10 μm, length l=30 μm). The microchannels are wet-etched in HF solution to a depth of 4 μm. Using standard photolithography and plasma etching (DRIE) on a 50nm layer of amorphous silicon (aSi) we obtain a well-controlled channel with nanodimensions. Finally, the device is sealed by anodic bonding to a second Pyrex wafer containing the powderblasted access holes. Solutions containing fluorescently labeled proteins are injected in inlet ports A and B. External negative pressure applied in outlet ports C and D allow driving the flows in each microchannel as well as the crossflow between these microchannels.

To investigate and characterize the hindered diffusion of proteins in our nanofluidic system, we used fluorescent wheat germ agglutinin (WGA, d_s=4 nm) with Alexa Fluor® 633. The FCS experiments have been performed with a ConfoCor microscope (Carl Zeiss) at a wavelength of 632.8 nm (HeNe).

4. Results and discussion

4.1. Steady diffusion

The steady diffusion across a nanochannel is schematically sketched in Fig. 2a. The channel initially contains a uniform concentration of solute c=0. At some time t_0, the concentration in the first microchannel is increased by injecting the proteins solution at a concentration c_0. Assuming that output microchannel concentration is remaining at zero (by rinsing with initial solution for example), a steady-state concentration profile is obtained when proteins have crossed the channel. Assuming no convective flow and using boundaries conditions (x=0 → c=$c_0 \cdot \Phi_{nC}$ and x=l → c=0), the concentration profile inside the 1D nanochannel at t=t_∞ can be expressed as:

$$c(x) = c_0 \cdot \Phi_{nC}\left(1 - \frac{x}{l}\right) \tag{5}$$

As presented in Fig. 2b, we measured the concentration gradient directly inside the nanochannel without any external differential pressure difference. The measurements were performed at high ionic strength (for c_i=10^{-1} M) in order to avoid interactions with the EDL.

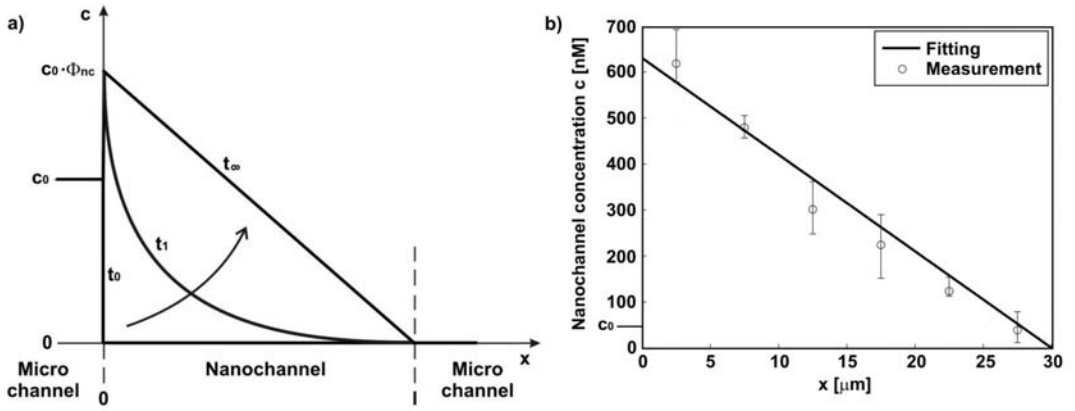

Fig. 2. (a) Principle and (b) measured concentration profile of WGA proteins diffusing from a first microchannel (filled with 50 nM WGA proteins in PBS) through a 50 nm high nanochannel of length l=30 μm into another microchannel filled only with PBS. No external pressure difference is applied. The fitting, based on equation 5, takes into account the equilibrium partitioning coefficient Φ_{nC} introduced in equation 4 (with Γ_{max}=7.22·10^{16} mol/m^2 and K=2.65 M^{-1}). The steady-state concentration profile is independent of the diffusion coefficient.

4.2. Steady diffusion and convection

When adding an external pressure difference ΔP, the concentration profile along the nanochannel can be deduced by writing a mass balance taking into account both diffusion and convective flow $u \neq 0$. Using the same boundaries conditions, the steady-state concentration profile inside the 1D nanochannel is determined as:

$$c(x) = c_0 \cdot \Phi_{nC} \left(e^{\frac{u}{D_{eff}}x} - e^{\frac{u}{D_{eff}}l} \right) \Big/ \left(1 - e^{\frac{u}{D_{eff}}l} \right) \tag{6}$$

Fig. 3 shows the concentration profile of 50nM WGA inside a nanochannel when an external pressure difference of $\Delta P = \pm 100$ mbar is applied. The curves are plotted using equation 6 with $D_{eff} = 7 \cdot 10^{-13}$ m^2/s.

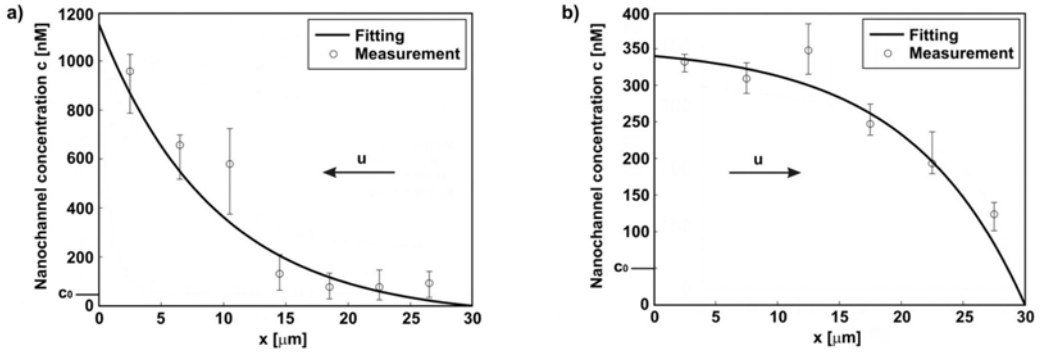

Fig. 3. Concentration profile of WGA proteins diffusing from a first microchannel (filled with 50 nM WGA proteins in PBS) through a 50 nm high nanochannel of length l=30 μm into another microchannel filled only with PBS. An external pressure is applied: (a) u=-70 nm/s and (b) u=70 nm/s. The fitting is based on equation 6 (with $\Gamma_{max} = 7.22 \cdot 10^{16}$ mol/m^2, $D_{eff} = 7 \cdot 10^{-13}$ m^2/s and K=5 M^{-1} for (a) and K=1.3 M^{-1} for (b)).

5. Conclusion

In conclusion, we have demonstrated that hindered proteins concentration and diffusion can be measured directly inside a nanochannel based on FCS. The models presented in this paper were used to predict concentration profile in nanoconfined spaces. We believe that it will help to get a better understanding of fundamental nanofluidic and molecular interaction physics.

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